**Case 34: Hereditary Periodic Fever Syndromes**

**Summary:** Generation of inflammatory response by the innate immune system leads to pain, redness, heat and swelling/edema in the infected tissue. Augments the killing of invading microbes by the front line macrophages and causes changes in the walls of local blood vessels that enable additional effector molecules and cells (neutrophils) to enter infected tissue from the blood. Inflammation also induces local blood clotting to make a physical barrier to the spread of infection through the bloodstream. Changes are induced by a variety of inflammatory mediators that are released by macrophages and other cells of the innate system as conseuqnece of their recognition of pathogens.

Pro inflammatory cyytokines inlcude IL1, IFNa, and IL6. IN addition to inducing tissue inflammation, they also induce fever. Fever is generally beneficial to the host defense because most pathogens grow better at lower temperatures and adaptive immune responses are more intense at elevated temperatures. IL1 and IL6 also enhance the production of innate proteins call acute phase reactants synthesized by the liver and released into the blood stream.

Because unregulated inflammation is harmful to the host, the production of pro inflammatory cytokines is tightly controlled so they are only produced in siginificant amounts in response to infection. Monocytes/macrophages, DCs, and nuetrophils detect the presence of pathogens by means of both cell-surface and intracellular receptors for microbial components. Intracellular bacterial infections of macrophages are detected by a family of intracellular proteins called NOD like receptors (NLRs). These include 2 subfamilies; NOD and NLRPs. NOD proteins detect bacterial cell wall muramyl dipeptides and respond by triggering a signaling pathway that terminates in activation of NFkB which switched on genes for pro inflammatory cytokines. NLRP subfamily are found in monocytes/macrophages and neutrophils where they act as sensors of cellular damage or stress as occurs on infection. Normally held inactive by accessory proteins in the cytoplasm. NLRPs are activated by a variety of stimuli produced by stressed cells such as toxins of s. aureus and l. monocytogenes. Leads to efflux of K from affected cells resulting in low levels of intracellular K. Leads to dissociation of inhibitory proteins from NLRP.

NLRP3 is an example of NLRP known as cryopyrin (because it was involved in inducing fever). In stressed cells, active NLRP3 forms part of a large protein complex called the inflammasome for its role in inducing inflammation. In the inflammasome NLRP3 is associated with protease caspase 1 which is activated within the inflammasome and is responsible for processing IL1 and IL18 proinflammatory cyotkines to give their active forms.

Group of clinical syndrome are called the autoinflammatory syndromes which manifest as inflammation in the absence of any evidnence of microbe infection. Characterized by episodes of fever accompanied by systemic and localized inflammation, affecting abdominal structures, joints, and the skin. Don’t involve self reactive T cells or self reactive autoantibodies so they are not autoimmune reactions (can also cause unexplained inflammation). Syndromes are driven by unchecked inflammatory responses. In normal patients the inflammatory cascade is tightly controlled by regulating the production of proinflammatory cytokines such as IL1. In contrast patients affected by autoinflammatory sundromes develop adaptive immune responses to stimuli such as infection or vaccination, but they are unable to control inflammation once its underway and inflammation may flare up in response to minor stimuli that would not elicit and inflammatory response in unaffected patients.

Autoinflammatory syndromes inlcude systemic onset juvenile rheumatoid arthritis and rarer diseases known as hereditary periodic fever syndromes (due to mutations in single genes). Defects in pathwyas that normally control the inflammatory response by regulating the production of pro inflammatory cytokines.

10 disorders are classified as hereditary periodic fever syndromes. Unified by their involvement of systemic and localized inflammation. Recurrent bouts of inflammation often don’t recurr at regular intervals. Cells affected include granulocytes, especially neutrophils and monocytes and macrophages. Chondrocytes are also affected in NOMID/CINCA.

Neonatal onset multisystem inflammatory disease/chronic infantile neurologic cutaneous and articular syndrome (NOMID/CINCA) is due to autosomal dominant mutation in NLRP3 gene and is an example of a hereditary periodic fever syndrome. Is one of 3 related but clinically distinct cryopyrinopathies among the hereditary periodic fevers which are characterized by excessive production of IL1b. Effects of excess IL1 include fever, rash, neutrophilia, high levels of platelets (thrombocytosis) and production of acute phase reactants. Other crypopyrinopathies (CAPS) are Muckle Wells syndrome (intermediate phenotype, urticaria and hearing loss) and familial cold autoinflmmatory syndrome (mildest, non purirtic urticaria, arthritis, chills, fever, and leukocytosis after exposure to cold) and all 3 result from mutations in the cryoporin encoding gene NLPR3. Mutants are constitiveively active and are hyperresponsive to microbial antigens or to physical stimuli such as cold leading to increased caspase 1 activation and exaggerated secretion of IL1 and IL18 (increased processing and secretion). Phenotype depends on modifying effects of other genes and the environment.

NOMID/CINCA patients have poor growth, neonatal onset intermittent fevers associated with persistent urticaria, arthritis like symptoms/arythropathy, and CNS involvement (papilledema-swelling of optic disc where sensory fibers from retina exit the eye, increased numbers of inflammatory cells in the CSF causing a chronic meningitis, sensorinureal hearing loss due to defect in nerve conduction or perception by the brain of auditory impulses). Also presents with dysmorphic facial features including frontal bossing, saddle nose, and long philtrum of upper lip. Enlarged spleen and liver with distended abdomen. Responseive to IL1R antagonist anakinra.

Other hereditary periodic fevers: familial Mediterranean fever is the most commons and is associated with mutations in MEFV which encodes pyrin. TNFR associated periodic syndrome (TRAPS) presents with longer episodes of fever, ab pain and rash due to mutations in the gene for TNFR 55kDa subunit (TNFRSF1A) leading to excess TNFa in the circulation. Hyperimmunoglobulinemia D with periodic fever syndrome has elevated levels of IgD with attacks leasting 7 days and recur every 4-8 weeks. Mutations in PSTPIP1 encoding the CD2 binding protein 1 (pyrin interacting protein) are associated with pyogenic arthritis, pyoderma gangrenosm and acne (PAPA). Mutations accentuate the interaction between pyrin and CD2BP1 and sequestration of pyrin results in excess secretion of IL1.

One of the most serious long term complications of the period fever syndromes is development of systemic amyloidosis which occurs when misfolded fragments of serum amyloid (acute phase reactant) are deposited in tissues. Kidney, GI tract, adrenals, spleen, testes and lung most often affects but liver, heart, and thryoid can also be involved. Amyloidosis occurs most commonly in patients with familial Mediterranean fever but can also affect paitents with TRAPS, Mucle Wells or NOMID/CINCA. Treatment with colchicine and TNFa inhibitors and IL1R anatgonist anakinra can help prevent amyloidosis.

Other inflammatory syndromes are Blau syndrome (uveitis, synovitis, rash, cranial neuropathies, frequently associated with Crohn’s). Due to AD mutaions in NOD2 that may interfere with binding of NOD2 to bacterial cell wall muramyl peptides and subsequet signaling through NFkB. Deficency of IL1 R antagonist (DIRA) leads to unopposed action of IL1 and presents as neonatal onset osteomyelitis, periostitis, and pustulosis (severe rashes and bone deformities) (AR for ILI1N). LPIN2 AR mutations leads to multifocal osteomyelitis associated with anemia and skin inflammation (chronic recurrent multifocal osteomyelitis and congenital dyserythropoetic anemia).

**Case:** sensorineural hearing loss in both ears at birth, 1 week developed intermittent fever accompanied by irritability (suspected severe bacterial infection), WBC was elevated, CSF showed high WBC count but no bacteria could be cultured, treated with antimicrobial drugs but fevers continued and no cause could be found, diffuse raise red itchy rash all over his body (urticaria), intermittent feverish, episodes of fever and irritability with no apparent source contibutes throughout the first year and his growth and development seemed slow, progresive hearing loss, prominent forehead and saddle shaped nose, enlarged liver and spleen, arthritis in the knees, increased erythrocyte sedimentation rate (due to increased concentration of certain blood proteins including acute phase reactants including elevated C reactive protein), txt with anakinra normalized growth and development and blood markers for inflammation normalized

Diagnosis of NOMID/CINCA made based on fever, rash, arthritis, hearing loss, and irritability. Genetic testing revealed mutation in NLRP3.

**Treatment:** anakinra (recombinant human IL1 receptor antagonist)

**Describe characteristics of autoinflammatory syndromes that include Crohn’s diseases and systemic-onset juvenile rheumatoid arthritis, as well as the rarer diseases due to mutations in single genes, that are collectively known as hereditary periodic fever syndromes.**

**Explain that autoinflammatory syndromes appear to result from poor regulation of inflammatory responses, and are not due to the actions of autoreactive antibodies or autoreactive T cells.**

**Describe the steps of the innate response that leads to inflammation (Fig 7.1).**

Innate immune mechanisms establish a state of inflammation at sites of infection. Pathogens penetrating the skin of mucosal epithelium invade underlying CT. They then activate resident effector cells such as macrophages to secrete chemokines, cytokines, and other inflammatory mediators which trigger that events leading to inflammation (vasodilation, increased vascular permeability allow fluid, protein and inflammatory cells to leave blood and enter tissue). Cells primarily attracted to inflammaed tissues are monocytes/macrophages and neutrophils which contribute to enhacing and sustaining the inflammatory state.

**Explain the activity of the proinflammatory cytokine, IL-1, and its stimulation of IL-6 production, which leads to acute phase protein production by liver cells and increased production of platelets (Fig 7.2).**

IL1 is secreted by many cell types including monocytes/macrophages to enter circulation and trigger IL1R on hypothalamic vascular network, resulting in synthesis of cyclooxygenase 2 which increases prostaglandin E2 in the brain to ride thus activating thermoregulatory center for fever production. In the periphery IL1 activates receptors in the endothelium resulting in rashes and production of IL6. Circulating IL6 stimulates liver cells to synthesize acute phase proteins which cause an icnreased erythrocyte sedimentation rate. IL1 also acts on bone marrow to increased mobilization of granulocyte progneitors and mature neutrophils resulting in peripheral neutrophila. Also indirectly increases IL6 induces platelet production resulting in thrombocytosis and causes a decreased response to erythropoietin resulting in anemia.

**Explain that defects in several distinct genes can cause a periodic fever syndrome.**

**Explain that uncontrolled production of IL-1 can lead to periodic fever syndrome, and that in the clinical case this can result from constitutive activation of cyropyrin, leading to lack of control of the activity of caspase-1 (the protease needed to process IL-1 into a mature form).**

**Explain that cryopyrin and caspase-1 are part of inflammasomes that are found in neutrophils and monocytes/macrophages, and normally only become activated during infection.**

NLRPs sense cellular damage and activate processing of pro inflammatory cytokines. Under normal conditions the LRR domain of NLRP3 associated with cytoplasmic proteins to prevent dimerization via the LRR region. When cells are injured or put under stress, the efflux of K ions triggers the dissociation of cytoplasmic proteins from NLRP3 allowing dimerization. The pyrin domains of NLRP3 recruit complexes of the adaptor protein PYCARD which associate with caspase 1 proenzyme through their CARD C domains. Aggregation of the proenzymes cause them to undergo autoactivation via proteolytic cleavage to form active caspase 1. Active caspase 1 cleaves the proprotein forms of pro inflammatory cytokines IL1 and IL18 to release the mature cytokines which can then be secreted.

Caspase 1 is required to process the precursor forms of IL1 and IL18 to produce mature active cytokines that can be secreted. IL33 also required processing by caspase 1 (IL1 like cyotkine that signals through ST2 to activate NFkB and MAPKs to drive production of Th2 cytokines such as IL4, 5, and 13). Would also see high levels of cytokines induced by IL1 such as IL6.

**Describe the action of anakinra.**

Recombinant protein that competitively blocks the binding of IL1 to the receptor to mitigate the effects of excess IL1 products in cryopyrinopathies. Pretreatment before exposure to cold has been shown to prevent development of symptoms and elevation of acute phase reactants in patients with familial cold autoinflmatorry syndrome and also improves symptoms and corrects biochemical abnormalities in Muckle Wells syndrome. In NOMID/CINCA patients there has been resuliton of fever, rash, and uveitis (inflammation of middle layer of eye uvea consisting of the choroid, iris and cilary body) and relief of excessive pressure exerted by the CSF. Used in patients with CAPS or DIRA, RA, sJIA.

Cholchicine: inhibits assembly of MTs in cells by binding t tubulin subunits. Thought to act mainly by inhibiting the neutrophil respose. Inhibits MT dependent processes in the cell including division of neutrophil precurosrs and secretion of pro inflammatory mediators by mature neutrophils. Also shown to module production of chemokines and pro inflammatory prostanoids (prostaglandins and LTs) by neutrohpils and inhibit neutrophil adhesion to the endothelium (needed for migration out of the blood and intro tissues). Also been shown to block IL1 secretion. All are antiinflamtory actions.

**Case # 35: Systemic-Onset Juvenile Idiopathic Arthritis (sJIA)**

**Summary:** Proinflammatory cytokines such as IL1b, TNFa, and IL6 elicit inflammatory effects leading to aberrant thermoregulation, metabolism, hematopoiesis and tissue inflammation.

Systemic onset juvenile idiopathic arthritis (sJIA) is an autoimmune condition consisting of arthritis along with systemic features such as high spiking fevers once or twice each day (body temp is normal or low between the fevers and patients feel well between the fever episodes), fatigue, rash (flat, salmon-pink, evanescent, tends to appear at same time as the fever) and enlargment of lymph nodes and spleen. Approximately 10% of kids with JIA initially show the systemic symptoms. No association with autoantibodies. Have increased secretion of IL1B unlike other forms of arthritis. sJIA seems to have more in common with inherited autoinflmmatory conditions than with other types of arthritis. In the active state, persistent activation of phagocytes and upregulation of inflammatory cytokines is seen. Polymorphisms in the promoters and coding sequences for the genes encoding IFNa, IL6, and macrophage inhibitory factor (MIF) have been associated with sJIA, but the inciting cause of sJIA remains unknown (don’t know the causitive gene).

Fever inducing IL1 drives IL6 production. IL1 has a very short half life, however IL6 levels can be measured and they correlate with fluctuations in the tmperature curve. The rash may be affected by some of the same inflammatory mediates as well as the fever itself. Rash tends to be more prominent with heat, including elevated room temperature. Can also be brough out after rubbing the skin or after trauma to the area.

Difficult to diagnose. Lab tests give nonspecific results and many of the manifestations (rash and fever) are common with childhood infections. Arthritis may be falyed in presentation. Differential diagnosis includes infection and malignancy because in some cancers WBC counts are elvated while other cells types are decreased. Criteria have been established to distinguish kids with infections and other transient disorders (such as serum sickness) from kids with true sJIA. Must have arthritis and daily fever for 2 weeks or more plus at least one of the following: evanescent red rash, generalized lymph node enlargement, enlargmenet of liver or spleen, serositis.

Can exclude kids that only develop arthritis later in their presentation. Also excludes kids with family history or personal history of psoriatic arthritis and other types of acute anterior uveitis or spondyloarthropathy (arthritic diseases that affect spine and large joints of the leg in conjunction with inflammation of tendon and ligament insertion sites), arthritis in a HLA B27 positive male older than 6 yrs, or presence of IgM rheumatoid factor on 2 or more occasions at least 3 months apart.

Initial treatment usually involves NSAIDs along with corticosteroids. Long term outcomes are highly variable. Some patients will improve after initial treatment and don’t have further manifestations of the disease, wehreas up to 1/3 of patients have persistant disease. Long term treatment often relies on tranditional arthritis meds such as methotrexate or prolonged courses of corticosteroids.

Newere treatments target the elevated levels of TNFa and levels of soluble TNF receptors that are associated with sJIA disease severity. Inhibitors of TNFa have been used but the response rate to these meds is significantly lower than in other types of JIA. Other therapies rely on inhibition of IL1 and IL6. Increased IL6 levels have been observed in the serum and joint fluid of sJIA patients and these levels correlate to disease activity. Spikes in IL6 also parallel that fever curve. Tocilizumab is a humanized monoclonal antibody directed against the IL6 receptor (approved for the treatment of rheumatoid arthritis) and has shown promising results in sJIA refractory to TNF inhibitors and other convential arthritis meds. Binds to the IL6 receptor. Side effects include increased infection rate and elevation of serum cholesterol when taking tocilizumab.

Althought levels of soluble IL1 are probably not elevated in sJIA, this cytokine binds to various large serum proteins and its total amount may be increaeed. Serum from patients with sJIA have been shown to induce IL1 secretion as well as transcription of innate immune genes from peripheral blood mononuclear cells from healthy patients. Therefore, IL1 receptor antagonists such as anakinra (used for treatment of RA and pediatric rheumatologic conditions such as sJIA) have been effective in controlling the systemic manifestations of sJIA. Because IL1 is important in normal response to infection, higher rates of serious infections of infectious complications would be possible with this treatment.

**Case:** 8 yr old girl with normal health until she began developing a 2 week history of high reccurent fevers and fatigue (twice daily), found it difficult to get out of bed in the morning because she ached all over but was able to move better later in the day, pink rash on shoulders and arms when the fevers occurred, swelling of several joints (ankles, knees, wrists) along with pain upon movement and decreased range of motion, parvovirus serologies were negative, WBC count was high and hemoglobin was low, erythrocyte sedimentation rate was elevated, very high C reactive protein, ferritin was high (these 3 are inflammatory markers), peripheral blood smear appeared normal except for mild anemia and increased neutrophil count

Symptoms, PE and labs were consistent with juvenile idiopathic arthritis

**Treatment:** indomethacin to control joint pain and fevers, prednisone (steroids) to treat inflammation, when steroid dose was reduced the fevers, rash, and joint pain returned, methotraxate continued and symptoms didn’t reappear

**Describe the role of cytokines in regulating the immune responses. List the most important pro-inflammatory cytokines and describe the function of each of these cytokines.**

IL1b/IL6/TNFa

Liver: acute phase protein production (CRP, mannose binding lectin) leading to activation of complement and opsonization

Bone marrow endothelium: neutrophil mobilization and phagocytosis

Hypothalamus: increased body temperature to decrease microbe replication, increase Ag processing and increase adaptive response

Fat and muscle: protein and E mobilization ot increase body temperature

DCs: TNFa stimulates migration to LN and maturation to initiation adaptive response

**Describe the role of IL-1beta and its role in the inflammatory process. How is caspase 1 involved in regulation of IL-1beta cytokine function?**

IL1b is synthesized as an inactive precursor molecule and cleavage of pro IL1b by caspase 1 is required for its activation and secretion. Regulated at multiple levels including the cleavage step. Activation of caspase 1 is dependent on the inflammasome (multi protein complex activated by substances such as microbe components, vaccine adjuvants, pollutatns, amyloid, and uric acid crystals). Severeal monogenic autoinflammatory disorders have been identified where genes for inflammasome proteins are mutated. Disorders are characterized by recurrent fevers, elevation of inflammatory markers, and skin, joint, and organ inflammation.

**Describe IL-6 and its role in regulating Th17 cells. List cytokines produced by Th17 cells. What roles do these cytokines play in the immune response?**

IL6 is produced primarily by T cells and is important for T cell differentiation and proliferation, as well as the differentation of B cells into plasma cells. Th17 cells produce IL17 and TNFa as well as IL6 to have a role in inflammation and induction of autoimmune diseases. Excess IL6 induces symptoms of fever, anorexia and fatigue, as well as elevation of acute phase reactants such as CRP, serum amyloid A and fibrinogen.

**What are the most characteristic symptoms for Systemic-Onset Juvenile Idiopathic Arthritis (sJIA)? Since exclusion of serum sickness, psoriasis, and arthritis is necessary, describe the most unique symptoms for sJIA. Explain how mutations of TNF-alpha, IL-6 and MIF may be associated with sJIA. High levels of TNF-alpha receptor in blood are also associated with sJIA.**

**What is the association of rheumatoid factor, HLA-B27, and/or autoreactive T cells with arthritis? If any of these factors are present, know that this is evidence to exclude sJIA as a diagnosis.**

**Case 47: Toxic Shock Syndrome**

**Summary:** Superantigens directly activate a large number of T cells. Bacterial and viral proteins that bind as whole unprocessed proteins simultaenously to MHC II molecles outside the peptide binding groove and to certain Vb chains of TCRs. By engaging all TCRs that share the same Vb chains rather than a receptor that recognizes a peptide in the MHC II cleft, SAGs activate many more T cells and generate an amplified T cell response far greater than that caused by the typical peptide Ag. Can activate naïve T cells. Induce prolifeartion of lymphocytes from neonates and from the thymus because previous exposure to the Ag and expansion of the number of Ag reactive cells isn’t required. SAGs don’t require processing by accessory cells and are able to induce the proliferation of purified T cells in presence of paraformaldehydr treated monocytes which lack the ability to process Ag. Direct binding of a labeled protein to cells positive for MHCII or its co precipitation with MHCII molecules confirms it as as superantigen.

First bind with high affinity to MHC II molecules on APCs. As concentration of bound SAG increases, it will bind and cross link TCRs with the appropriate Vb specificity (doesn’t involve a chain). Cross linking activates both the T cells and the APC through signaling events downstream of the TCR and MHCII. Because of the high affinity for MHCII molecules, a very small amount of SAG results in intense T cell signaling. Each SAG is capable of binding to a limited group of Vb regions. TSST1 produced by s. aureus stimulates all the T cells that express Vb2 gene segment. As there is a limited repertoire of Vb gene segments, any SAG will stimulate between 2-20% of all T cells. Activation leads to massive secretion of IL2, IFNg, TNFa and lymphotoxin. Can also directly activate monocytes and DCs by crosslinking their MHCII molecules leading to rpaid and massive release of IL1, TNFa, IL6, CXCL8 and IL12. This is associated with an upregulation of B7 co stimulatory molecules and further amplifies T cell activation contributing to rapid onset of clinical symptoms.

This stimulation is not specific for the pathogen and doesn’t lead to adaptive immunity. It causes an excessive production of cytokines by the large number of activated CD4 T cells. Cytokine release causes systemic toxicity and supression of the adaptive immune response. Both effects contribute to the pathogenicity of microbes that produce SAGs.

Staph toxic shock syndrome is caused by TSST-1 superantigen by binding Vb2 on the TCR. Rapid onset of fever, rash, organ failure, and shock. Most cases are in menstruating women in their teens but cases occur in all age groups. Associated with localized s. aureus infection (subQ abcesses, osteomyelitis, and infected wounds), staph food posioning, or local colonization (vagina in this case). Tampons kept in for more than 12 hours soaked with menstral fluids can enhance growth of bacteria that are the source of superantigens (unlikely in this case because it was only in for 6 hrs.). Toxigenic strains of s. aureus can produce enterotoxins (enterotoxin B) as well as TSST1 which both act as superantigens. Other microbes produce SAGs.

Circulating lymphocytes from patients in the acute phase of TSS typically show a higher proportion of circulating Vb2 T cells than other Vb segments. As the illness resolves, there is gradual return to near normal proportions. Expansion can be measured by examining the surface expression of Vb2 TCs with IF using anti anti Vb2 Ab or by semiquantiative measurement of mRNA transcripts encoding the Vb2 TCR chain via RT PCR after stimulation with anti CD3 Ab and IL2.

Although T cells activated by a given SAG will have a common Vb region, they will differ in their specificity for conventional peptide antigens. Sequencing of TCRs will show the use of different D and J gene segments by the beta chains and a wide diversity of alpha chains and will encompass a wide varety of Ag specificities (conventional Ag will induce clonal expansion with T cells of identical D and J gene segments in their b chains and identical a chains). Because the pool of Vb T cells may contain autoreactive T cells, superantigens may trigger autoimmune disease.

Many of the manifestations are the result of massive and unregulated cytokine production triggered by activation of immune system cells. TSST-1 is more effective than LPS in inducing synthesis and secretion of IL1 and TNFa by monocytes. Is also a potent T cell mitogen for T cells and induces them to produce large amounts of cytokines such as IL2 and IFNg. IL1 and TNF a are critical in induction of acute phase response characterized by fever and production of IL6. IL1 and TNFa also activate vascular endothelium and together with IL2 increase vascular permeability with subsequent leakage of fluid from intravascular space into the perivasculate. Massive overproduction of TNFa resulting in toxic shock including edema and intravascular volume depletion leading to hypotension, shock, and MOF.

Liver injury may occur as result of decreased orgn perfusion during hypotension. Immunological mechanisms may also contribute. Hepatocytes express Fas. T cell activation and release of cyotkines results in upregulations of FasL on surface of circulating lymphocytes. Cross linking of Fas on hepatocytes by FasL on circulating lymphocytes results in triggering of apoptosis in hepatocytes. In addition, TNFa is also capable of triggering cell death resulting in liver injury.

S. aureus colonizes 25-50% of the population and nearly 50% of isolates produce superantigens. TSS is rare for several reasons. Staph cell wall contains PGs that bind to TLR2 and TLR6 on monocytes resulting in activation of NFkB causing an anti inflammatory response mediated by IL10 production and subsequent apoptosis of APCs. This aborts the amplification of the inflammatory response associated with TSS. Also, susceptibility to TSS correlates with poor Ab response to TSST-1. Usually individuals are exposed to bacterial numbers that are too low to produce dangerous levels of SAG but high enough to generate toxin specific Abs. Althought most healthy people have protective Ab titers to TSST-1, more than 80% of patients with TSS lack anti-TSST1 antibodies during acute illnes and most fail to develop anti TSST-1 Abs after convalescence. Could be due to an individuals inability to mount an antibody response to TSST-1 and staph enterotoxins or specific inhibition of such a response by the toxins. Also, certain HLA polymorphisms (HLA DQ allele) are associated with increased superantigen affinity for MHCII molecules and more severe clinical symptoms.

Because AB against staph and strep SAGS confer protection against SAG medited diseases and vast majority of donors of the plasma pool sused to prepare IVIG have Ab against TSST-1, IVIG is a potential treatment for TSS patients. IVIG should be used in combo with antibiotics and its efficacy in neutralizing superantigens can differ between preparations.

**Case:** 16 yr old girl with history of mild astham and allergic rhinitis, suddenly become feverish, general m. aches and dizziness, naseated and vomited, briefly lost consciousness, red rash on arms and spread rapidly to most of her body, alert but listless, HR and RR markedly elevated, BP was depressed, showed significant volume depletion, used a tampon that morning, WBCs were elevated with predominance of neutrophils and band forms (Immature neutrophils) indicating increased mobilization of neutrophils from the bone marrow, blood coagulation time was slightly prolonged and serum transaminase levels were raised (both consistent with abnormal liver function), vaginal culture positive for s. aureus,

**Treatment:** given cephalopsorin (ceftriaxone) along with IV fluid, developed petechiae and given oxacillin and clindamycin and cefotaxime and IVIG

**Update on material in book:**

Please note that the discussion on point 4 on page 255 (answers on 337) is a little misleading. Many individuals resistance to toxic shock syndrome is associated with the production of anti-TSST antibodies, which are formed in about 90% of normal adults. Of the persons without antibodies and who do develop toxic shock due to TSST-1, around 50% fail to make a specific antibody response, so remain susceptible. The others end up with an antibody response that is protective. This may be partly due to dose effects (e.g. early treatment and low inoculum may allow a protective immune response to develop before enough toxin is produced to saturate the susceptible subsets of T cells).

**Describe the roles of CD4, MHC class II, TCR, antigenic peptide, and CD3 in inducing activation of T cells in response to conventional antigens.**

**Explain that superantigen binds directly to MHC class II molecules and to the variable region of TCR, thus obviating the need for an antigenic peptide that is specifically bound by a TCR.**

**Explain that currently described superantigens all bind to the TCR V region.**

**Explain that different superantigens vary in their binding to different subgroups of TCR V and that these TCR subgroups differ by having distinct framework-encoding sequences within V[Do not bother to learn which toxins bind which subgroups!]**

**Explain that *Staphylococcus aureus* makes several different types of toxins, and these include the enterotoxins and toxic shock syndrome toxins which act as superantigens. Explain that Group A streptococci produce a superantigen that causes toxic shock syndrome.**

**Describe toxic shock syndrome as a disease that is associated with a low blood pressure and a non-petechial rash.**

**Explain that widespread activation of subsets of T cells (typically 2-20% of all T cells can be activated by a superantigen) leads to massive release of cytokines, including IL-1, TNF-, IFN and LT (lymphotoxin), and these cause the production of shock. Explain that activation of the T cells leads to their division and a large increase in cell number.**

**Explain that some superantigens activate macrophages and dendritic cells directly because they crosslink MHC class II molecules; this can occur because those toxins possess multiple MHC class II binding sites. This crosslinking can lead to release of proinflammatory cytokines from these antigen presenting cells (APCs).**

**Case 48: Lepromatous leprosy**

**Summary:** Mature naïve CD4 T cells emerge from the thymus and differentiate into effector CD4 T cells with different phenotypes. Th1 and Th2 cells develops from naïve T cells that are activated by pathogen Ags in the presence of different signals provided by APCs and the local environment. Distinguished by the types of cytokines that they secrete when they encounter their target cells. Th1 cells secrete IL2, IFNg and lymphotoxin. Th2 cells secrete IL4, IL5, and IL10. Th17 cells secrete IL17 and regulatory T cells also develop.

Selective production of Th1 cells enable the immune response to activate macrophaes and CMI whereas Th2 production biases the response toward Ab production only. Decision to become a type of T cells occurs during its first encounter with Ag and is dependent on cytokines. Th1 differentiation is dependent on IL12 and IFNg whereas Th2 differentation is dependent on IL4. Cytokines trigger pathways of signal transduction (mice deficient in STAT6 induced by IL4 will lack Th2 cells). Other factors are the amount of Ag present. Large amounts of Ag are usually presentd by DCs which produce IL12 and favor Th1 differneitation. Small amounts of Ag are presented by Ag specific B cells and induce Th2 differentation. Costimulatory molecules expressed by the APCs can also play a role with B7.1/CD80 favoring Th1 development and B7.2/CD86 favoring Th2 development.

Decision to differentiation occurs early in an adaptive response and the ability of pathogens to stimulate cytokines prudction by cells of the innate system has an important role in determining the subsequent course of the response. Microbes that invade or nonspecifically activate macrophages and NK cells (viruses, intracellular bacteria such as mycobacteria) induce cells to secrete IL12 and favor differentiation of IFNg secreting Th1 cells. Loop is amplified because IFNg favores Th1 development and blocks Th2 deveoopment. Il12 also enhances proliferation of Th1 cells but has no effect on Th2 cells because they don’t express the beta chain of the IL12 R.

Differentiation of Th2 cells is favored by pahtogens such as parasites that elicit IL4 production from cells such as mast cells, eosinophils, and thymus derived invarient NKT cells (express NK.1.1 marker and TCR of resitrcted Vb and invarient Va chain usage). Loop is amplified by Il4 and IL10 produced by Th2 cells. IL4 promotes Th2 cell development and IL10 blocks Th1 development.

Only 1 type of Th phenotype becomes dominants in the course of a reponse and it is difficult to shift the antigen specific response to the other. One reason is because the cytokine products of Th1 and Th2 cells are reciprocally inhibitory. The outcome of certain infections such as mycobacterium leprae is greatly influences by the type of T cell response that is elicited.

Classic clinical features of leprosy are cutaenous lesions, neropathologic changes, and deformities. Mycobacterium leprae colonizes macrophages and other host cells and multiplies within them. Bacteria are protected within macrophages from attack by Ab and can be eliminated only when their host macrophages are activated and produce increased amounts of NO, O radicals, and other microbicidal molecules. Grows best at 30C so that is why lesions are usually on extremities (hands, ears, butt). Doesn’t grow in culture. In ttisue see numerous small dark red dots inside macrophages. Clinical symptoms vary depending on the type of immune response to the bacteria. Importance of Th1 dervied IFNg is containing mycobacterial infections is shown by the observation that infants with genetic defects in the IFNg receptor die from disseminated mycobacterial infections.

Tuberculoid: CMI Th1 response (see IL2, IFNg, LT), results in macrophage activation with efficient killing of intracellular bacteria, localized tissue damage and milder clinical picture, bacterial are containing within granulomas and propoagate poorly, usually with subsequent min. tissue damage, well controlled by Th1 cells that activate macrophages, low infectivity, peripheral n. damage, normal serum Ig levels, normal T cell responsiveness specific to M. leprae Ag

Lepromatous form: Th2 response predominants (see IL4, IL5, IL10) leading to vigorous but inefficective Abr esponse and dissemination of the bacteria to other sites in the body resulting in further tissue destruction and symptom aggravation, bacteria propogate rapidly resulting in extensive tissue damage, grow uncontrolled in macropjages, in late stages there is severe damage to CT and peripheral NS, high infectivity, bone, cartialge and diffuse n. damage, hypergammaglobulinemia (humoral immune response drived by Th2 cells, cytokines lead to enhanced Ig production, IL4 induces switching to IgE and increased IgG4 and IgE production, Il10 stimulates production of IgG1 and IgG3, Il5 stimulates global Ig production), low or absent T cell responsiveness with no response to M. leprae Ag.

Neurologic damage has 2 main causes. Arise from bacterial multiplical within Schwann cells that form the insulating myelin sheath around axons leading to interferance of normal condution of nerve impulses along the axon. In the tuberculoid form nerve damage also arises from formation of granulomas and inflammation of tissue surrounding the nerve. Damage results in dysfunctional nerve terminals causing decreased sensation and eventually loss of motor function.

Nosebleeds are common and are due to large amounts of bacteria in the nasal tissue with extensive involvement of nasal mucosa leading to congestion and breakage of blood vessels.

Th2 response also influences infection: Ab bind to Ag on surface of infected cells and interfere with action of CD8 T cells. CD8 cells can also response to Ag by secretng cytkines. In Lepromateous they have CD8 cells that supress Th1 response by making IL10 and LT. IL10 inhibits Th1 development and inhibits cytkine release from macrophages and their abaility to kill internalized microbes. LT also inhibits intracellular killing of macrophages leading to decreased production of IL12, fever Th1 cells and more Th2 cells. In patients with tuebrculoid they lack suppressor CD8 cells and thus make a viorous Th1 response leading to macrophage activation and destruction of bacteria.

**Case:** 18 yr old girl from Columbia, gradual loss of sensation on backs of her hands, hypopigmented lesions on both arms that progressively became worse, loss of eyelashes and eyebrow hairs, recurrent nosebleeds, history of mild asthma, multipl hypopigmented macules (coin sized raised lesions with ill defined borders) on skin along with cutaneous nodules predominantely on elbows, wrists, hands and showed traces of dried blood, also on knees, ears, and butt, neuro exam showed ecresed response to pinprick on outer edges of the R and L hand and R 4th and 5th fingers, flexion contractur of fingers on both hands so she couldn’t straighten these fingers completeley, blood tests were normaly, biopsy of lesion showed numerous acid fast bacilli in clumps, hematoxylin and eosin stain of lesion tissue showed numerous Virchow’s cells (highly vaculoated macrophages known as foam cells) and few lymphocytes, delayed hypersensitivity skin tests with intradermal injections of candida mumps and tuberculin Ag showed no reaction, serum IgG was slightly elevated

Diagnosis of lepromatous leprosy made on basis of presence of acid fast bacilli in biopsy and progressive neurological symptoms

Prone to asthma because Th2 response leads to increased production of IL4 and IL10. When she encounters a new Ag her immune system will be flushed with Il4 triggering a Th2 response to the Ag leading to IgE response. Asthma is a Th2 driven condition.

**Treatment:** dapsone, clofazamine, rifampin

Would like to swtich to a Th1 response. Cytokines with the potential to inhibit Th2 and induce Th1 are IL2, IFNg, and IL12. Local injection of IFNg has been shown to lead to partial reversal of anergy and reduction of lesions (also useful in leishmaniasis, visceral form is skewed to Th2 response in contrast to the cutaneous form accompanies by Th1 response). IL 12 might also be beneifical because it can induce Th1 cells and doesn’t activate Th2 cells.

**Explain that leprosy is a disease caused by infection with *Mycobacterium leprae*, that it has a predilection for infecting nerves, and that the bacterium has not been cultivated *in vitro*.**

**Describe the different types of immune response to leprosy and how these lead to distinctive clinical appearances of the disease.**

**Explain that a strong Th1-mediated response characterizes tuberculous leprosy, where granulomas are formed and few bacteria are detectable in tissues. The strong inflammatory response can cause significant nerve damage and paralysis.**

**Explain that a Th2-mediated response does not control bacterial numbers, and these persistent bacteria cause tissue destruction and loss of elasticity; these conditions are associated with lepromatous leprosy.**

**List the cytokines that characterize Th1 and Th2 responses (see figure 30.5).**

Naïve CD4 T cell that is uncomitted first response to their Ag via MHCII by making IL2 and proliferating, then differentiation into immature effector Th0, then to distinct function subsets:

Th1: induced by IL12 and IFNg (T bet and STAT4), produce IFNg, IL2, and LT, activates macrophages, induces B cells to produce opsonizing antibodies, IFNg prevents Th2 differentiation, In

Th2: induced by IL4 (GATA3 and STAT6), produces IL4, IL5, and IL10, activates B cells to make neutralizing Ab, various effects on macrophages, IL4 and IL10 prevent Th1 cells differentiation

Th17 cells: induced by TGFb and IL6 (ROR-gT and STAT3), produce IL17, IL21, IL22, protects against intracellular pathogens (candida), involved in autoimmune manifestations

T reg: induced by TGFb and IL2 (FoxP3), inhibited by IL6 and IL21, produces TGF b and IL10, immunsupressive properties

**Explain that lepromatous leprosy is associated with hypergammaglobulinemia.**

**Explain that strong DTH skin test reactions to recall antigens (such as mumps) and to lepromin (derived from leprosy tissues) is characteristic of tuberculoid leprosy, but skin test anergy is common in lepromatous leprosy.**

Absence of delayed type hypersensitivity to a wide range of antigens unrelated to M. leprae is called anergy (not the same as T or B cell anergy). In tuberculoid leprody, there is a dtrong delayed type hypersensitivty to M. leprae and no angery. Existance of anergy in the lepromatous form but no in the tuebrculoid form is probably due to the presence of regulatory CD8 T cells in the lepromatous form. The CD8 cells secrete IL10 and LT and suppress Ag presentation by macrophahges and suppress T cell responses to other unrelated Ags. IL10 and LT suppress M. leprae T cells and also neighboring T cells leading to global hyporesponsiveness. Many patients with lepromatous form the unresponsiveness is confined to M. leprae and responses are made to other Ag. Other pathogens use IL1- to produce anergy (EBV produces vIL10 that is homolgous to human IL10, mealses binds to CD46 on monocytes and inhibits IL12 production).

**Case 52: Drug –induced serum sickness**

**Summary:** IV administration of a large dose of soluble Ag can evoke in some patients a type III hypersensitivity reaction or IC disease (effectors are complement and FcR cells). Ag administration produces a rapid IgG response and the formation of Ag:Ab complexes that can activate complement. As a result of the large amount of Ag present and the rapid IgG response, small ICs form in the conditions of Ag excess. Unlike large ICs that are formed in conditions of Ab excess which are rapidly cleard by phagocytes, small ICs are sometimes too small to precipitate and taken up by endotheial cells and become deposited in tissues. Local activation of complement by these immune complexes provokes localized inflammatory response and damage to blood vessels and other tissues.

Experimental model is the Arthus reaction in which a subQ ingection of a large dose of Ag evokes a rapid IgG response in patients who have already made IgG Ab against the allergen. The activation of complement by the complexes generate C3a/C5a which is a potentent stimulator of histamine release from mast cells and C5a which is an active chemokine. The local endothelial cells are activated by interactions in the blood vessels between the ICs, complement, and circulating leukocytes and platelets. They upregulate expression of adhesion molecules (selectins and integrins) which fiacilitates the emigration of WBCs from the blood and the initiation of local inflammatory reaction with increased fluid and protein release, phagocytosis. Platelets also accumulate at the site and cause blood clotting. Small vessels become plugged with clots and burst producing hemorrhage in the skin. Takes 1-2 hrs.

When Ag (such as antibiotics) is injected IV, the ICs can be deposited at a wide range of sites. When in synovial tissue the resulting joint inflammation produces arthritis. In the kidney glomeruli they cause glomerulonephritis, in the endothelial of BVs of the skin and other organs they produce vasculitis. Fever due to activation of complement and phagocytes.

Although horse serum is no longer used, other forgein proteins are still administered such as antitoxins to snake venom produced in animals and mouse mabs. Commonest cause of serum sickness today are antibiotics such as penicillin and its derivatives that act as haptens. They bind to host proteins that serve as carriers and can eleict a rapid and strong IgG response. Self limiting disease that terminates as the immune response moves into a zone of Ab excess (remove the foreign protein). Can be fatal if it provokes kidney shutdown or bleeding in critical areas such as the brain. Course can be ameliroated by anti inflammatory drugs (prednisone and antihistamines). This is a reaction that can appear on first encounter with the Ag if it is long lived and given in a suffiencetly large dose.

**Case:** 12 yr old boy treated with IV ampicillin and penicillin for treatment of pneumococcus (fever, cough, SOB, pale, dehydratied, increased RR and HR, crackles, opaque CXR over entire lower lobe of left lung, lobar pneumonia, increased WBC with increased immature neutrophils), no history of penicillin allergy, on 9th day in hospital developed puffy eyes, welts that looked like large hives on his abdomen, treated with oral antihistamine and penicillin was discontinued, 2 hrs later developed tight feeling in throat, swollen face and widespread urticaria, wheezing heard all over lungs that responseded to albuterol inhalation, developed fever and swollen and painful ankles and rash become more generalized and spread to trunk, back neck and face and become confluent in palces, reddneded eyes due to inflammed conjunctivae and swelling around mouth, cervical axillary and inguinal lymph nodes on both sides were enlarged, spleen was enlarged, ankles and knees were swollen and tender and too painful to move, alert, raise WBC count and predominance of lymphocytes, plasma cells detected in blood smear, ESR was elevated, serum complement levels and C1q and C3 were decreased, purpuric lesions caused by hemorrhading of small blood vessels under the skin on feet and ankles, agitated and periods of disorientation, negative CT and CSF, electroencephalogram had pattern that suggested diminished circulation in P part of brain, WBC and EST continuted to rais, red cells and protein in urine, skin biopsy of purpuric area showed moderate edema around capillaries and dermis as well as perivascular infiltrates of lymphocytes in deeper dermis, IF showed seposition of IgG and C3 in perivascular areas, txt with prednisone and 2 weeks later had no IgE against penicillin or ampicillin as etected by hypersensitivty skin test and RAST

Hives and edema caused by activation of complement generated C3a which releases histamine from mast cells and causes hives. Swelling around mouth and eyes is a form of angioedema. Confusion and neuro symtpoms because he developed vasculitis in the small BVs of the brain which compromised oxygen delivery. Red cells and protein in urine indicated inflammation of BVs in glomeruli. Purpura in feet and ankles indicates hemorrhage from BVs in skin that are inflammed and have become plugged with clots. Biopsy showed deposition of IgG and C3 around BVs suggesting that an immune reaction was taking place.

Biopsy of a LN would show massive follicular hyperplasia, polyclonal B cell activation and many mature plasma cells in the medulla. Massive B cell activation in LN leads to overflow of plasma cells from the medulla into the efferent lymph. Its very rare to find plasma cells in the blood (enter via the thoracic duct). Enlargement of spleen due to hyperplasia of the white pukp. Some plasma cells probably enter the blood from the hyperplastic follicles in the spleen.

Brisk acute phase response was caused by IL6 and IL1 released from monocytes that have been activated by uptakes of ICs. Consists of canges in liver protein synthesis. Albumin and transferrin synthesis drops and synthesis of fibrinogen, CRP, amyloid A and glycoproteins is upregulated. Part of innate immunity.

Serum C1q level decrease almost always indicates complement consumption by ICs via the classical pathway (in hereditery angioedema the C1q level is normal because complement is activated due to a defect in an inhibitor). C3 was also low which indicates complement consumption.

Skin test was negative because he didn’t have IgE antibiodies against penicillin as confirmed by negative RAST test. Serum sickness is caused by complement fixing IgG antibiodies.

**Treatment:** benadry and naproxen (NSAID), anti inflammatory corticosteroid prednisone, avoid Ag

**Explain that when a fixed amount of antibody is mixed with increasing amounts of antigen (as in Figure 35.2), different amounts of antibody can be expected to be precipitated and that, at antigen excess, antibodies will remain soluble in the form of small immune complexes. (This is because cross-linking of multiple antigen and antibody molecules to form very large complexes is prevented by antigen saturating the antibodies.)**

**Define anaphylatoxin. Describe how activation of the classical complement pathway by binding of immune complexes by C1q leads to the generation of C5a and C3a, which are anaphylatoxins. In addition, recognize that C5a is a potent chemoattractant leading to PMN chemotaxis.**

**Define hapten. Recognize that serum sickness can follow an antibody response to large amounts of foreign antigen present in the plasma. Recognize that the foreign antigen may be mimicked by host proteins that have bound a foreign hapten (e.g. penicillin), thereby inducing an anti-hapten antibody response.**

Elicts an immune response when bound to a carrier.

**Recognize that serum sickness was, in the earlier 20th century, mainly associated with use of horse serum antibodies as therapy (e.g. anti-tetanus toxin, anti-diphtheria toxin, anti-scarlet fever).**

**Explain that disease symptoms of serum sickness follow the widespread production of soluble immune complexes (Figure 35.7).**

**Recognize that serum sickness symptoms include fever, vasculitis, arthritis and nephritis (with proteinuria), and that these result from the inflammation associated with deposition of immune complexes in small blood vessels.**

**Explain that Type I hypersensitivity reactions follow the interaction of cytotropic antibodies on mast cells and basophils with antigen, thus Type I hypersensitivity does not describe the hypersensitivity responsible for serum sickness. Explain that serum sickness-associated hypersensitivity is mediated by IgG antibodies that, when complexed to antigen, are capable of fixing complement, and that any mast cell degranulation that occurs in serum sickness is caused by generation of anaphylatoxins (C3a and C5a).**